Vol. 465: 33–52, 2012
doi: 10.3354/meps09879

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published September 28

Pigment-based chloroplast types in dinoflagellates

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ABSTRACT: Most photosynthetic dinoflagellates contain a chloroplast with peridinin as the major carotenoid. Chloroplasts from other algal lineages have been reported, suggesting multiple plastid losses and replacements through endosymbiotic events. The pigment composition of 64 dinoflagellate species (122 strains) was analysed by using high-performance liquid chromatography. In addition to chlorophyll (chl) *a*, both chl c_2 and divinyl protochlorophyllide occurred in chl *c*-containing species. Chl c_1 co-occurred with chl c_2 in some peridinin-containing (e.g. *Gambierdiscus* spp.) and fucoxanthin-containing dinoflagellates (e.g. *Kryptoperidinium foliaceum*). Chl c_3 occurred in dinoflagellates whose plastids contained 19′-acyloxyfucoxanthins (e.g. *Karenia mikimotoi*). Chl *b* was present in green dinoflagellates (*Lepidodinium chlorophorum*). Based on unique combinations of chlorophylls and carotenoids, 6 pigment-based chloroplast types were defined: Type 1: peridinin/dinoxanthin/chl c_2 (*Alexandrium minutum*); Type 2: fucoxanthin/19′-acyloxy fucoxanthins/4-keto-19′-acyloxy-fucoxanthins/gyroxanthin diesters/chl c_2, c_3, monogalactosyl-diacylglycerol-chl c_2 (*Karenia mikimotoi*); Type 3: fucoxanthin/19′-acyloxyfucoxanthins/gyroxanthin diesters/chl c_2, c_3 (*Karlodinium veneficum*); Type 4: fucoxanthin/chl c_1, c_2 (*K. foliaceum*); Type 5: alloxanthin/chl c_2/phycobiliproteins (*Dinophysis tripos*); Type 6: neoxanthin/violaxanthin/a major unknown carotenoid/chl *b* (*Lepidodinium chlorophorum*). While plastids with peridinin, and probably those with chl *b*, originated by secondary endosymbiosis, the other chloroplast types were obtained through tertiary endosymbiosis. Chloroplast types corresponded with evolutionary lineages within dinoflagellates. Caution must be observed when only peridinin is used for tracking photosynthetic dinoflagellates in field samples. The additional marker pigments offer oceanographers greater power for detecting dinophytes in mixed populations.

KEY WORDS: Dinophyta · Chlorophyll *c* pigments · Novel fucoxanthin-related pigments · Gyroxanthin diester pigments · Chemotaxonomy · Dinoflagellate chloroplast types · Plastid origin Oceanography

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INTRODUCTION

Dinoflagellates are a diverse trophic group of alveolate protists with around 50% of autotrophic organisms with a distinct grade of mixotrophy (Jeong et al. 2005). Most photosynthetic species contain a chloroplast surrounded by 3 membranes with chlorophyll (chl) c_2 and peridinin as major accessory pigments (Jeffrey et al. 1975).

Several features make autotrophic dinoflagellates unique: (1) the presence of a water-soluble light-harvesting protein, i.e. the extrinsic peridinin-chl *a*-protein located in the chloroplast thylakoid lumen (Hofmann et al. 1996, Hiller 1999), which occurs in several species together with the ubiquitous intrinsic peridinin-chl *a*-chl c_2 membrane-bound light-harvesting protein (Macpherson & Hiller 2003); (2) the presence of a proteobacterial form of the key enzyme in photosynthesis, the ribulose bisphosphate carboxylase/oxidase (Rubisco), the so-called form II Rubisco (Morse et al. 1995, Rowan et al. 1996) which is nuclear-encoded; (3) the gain of tertiary plastids in certain autotrophic dinoflagellates (Saldarriaga et al. 2001).

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Some photosynthetic dinoflagellates are toxin producers, and the monitoring of these species is the main target of harmful algal bloom (HAB) programmes around the world (e.g. GEOHAB: www.geohab.info). In consequence, taxonomists and oceanographers have sought procedures for the rapid detection and identification of toxic species (see Hallegraeff et al. 2003). Among these techniques, the use of chloroplast pigments as fingerprints of phytoplankton taxa (Jeffrey & Vesk 1997, Jeffrey et al. 1999) constitutes a useful approach to study the plastid origin and taxonomy of dinoflagellate species. In this context, the presence of peridinin, an allenic trinor carotenoid (i.e. having a C_{37}-skeleton instead of the common C_{40}-xanthophylls), has been considered a distinctive feature in photosynthetic dinoflagellates (see Bjørnland 1990, Liaaen-Jensen 1998). Although the occurrence of peridinin-containing chloroplasts is widespread within autotrophic dinoflagellates, the occurrence of fucoxanthin-containing dinoflagellates was observed in earlier studies (see Jeffrey et al. 1975). Later, several dinoflagellates with different pigment patterns were detected: first, a marine dinoflagellate containing 19′-hexanoyloxyfucoxanthin as the main carotenoid (Tangen & Bjørnland 1981), then a chl *b*-containing 'green' dinoflagellate (Watanabe et al. 1987), which was further described as *Lepidodinium viride* (Watanabe et al. 1990), and finally autotrophic *Dinophysis* species with cryptophycean-type phycobiliproteins (see Geider & Gunter 1989, Hewes et al. 1998). Information on chloroplast pigments has usually been reported in new descriptions of dinoflagellates (e.g. Fraga et al. 1995, 2008, 2011, Elbrächter & Schnepf 1996, Bolch et al. 1999, Daugbjerg et al. 2000, Montresor et al. 2003, de Salas et al. 2003, 2004, 2005, Garcés et al. 2006, Tillmann et al. 2009, Sampedro et al. 2011).

Methodological improvements in high-performance liquid chromatography (HPLC) pigment analysis (revised by Jeffrey et al. 1999, Garrido & Zapata 2006) have enabled us to detect new chl *c* pigments and fucoxanthin acyloxy derivatives and to obtain a more accurate distribution pattern of known pigments. An example of this is the description of 8 pigmentary types in Haptophyta (Zapata et al. 2004).

Here we report the pigment composition of 64 dinoflagellate species (122 strains) obtained by HPLC. Six pigment-based chloroplast types are described and compared with other algal lineages. Pigment diversity found in photosynthetic dinoflagellates provides clues both for inferring phylogenetic relationships and tracing the distribution and abundance of dinoflagellates in coastal and open-ocean waters.

MATERIALS AND METHODS

Algal cultures

Dinoflagellate cultures were obtained from the Culture Collection of the Instituto Español de Oceanografía (CCVIEO, Vigo, Spain), the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA — formerly CCMP — Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine, USA) and the CSIRO Collection of Living Microalgae (CCLM, Hobart, Australia). Most cultures were grown in L1 medium (Guillard & Hargraves 1993), with the exception of the CSIRO strains which were grown in GSe medium (Blackburn et al. 1989). The species, strain numbers, collection site and isolator are listed in Table S1 in the supplement at www.int-res.com/articles/suppl/m465p033_supp.pdf. Light irradiances were 60 to 90 µmol quanta m^{-2} s^{-1} on 12:12 h light:dark cycles for all strains except *Dinophysis* (150 µmol quanta m^{-2} s^{-1}). Cultures of *Dinophysis* species (*D. acuminata*, *D. acuta*, *D. caudata* and *D. tripos*) were established in diluted (1/20) L1-Si medium by feeding them the phototrophic ciliate *Mesodinium rubrum* (= *Myrionecta rubra*), fed with the cryptophyte *Teleaulax* sp. (Park et al. 2006).

Sample preparation

Cultures were examined by light microscopy before HPLC pigment analysis to ensure proper identification and that the cells were healthy and without malformations. Cells were harvested 2 to 4 h into the light cycle from cultures actively growing a few days after being transferred. Due to the great diversity of species being analysed, variable volumes of culture (typically 10 ml), depending on the species, were filtered onto Whatman GF/F filters under reduced pressure until some colour was observed on the filter. Filters were frozen immediately at –20°C.

Pigment extraction

Frozen filters were extracted under low light in polytetrafluoroethylene (PTFE)-lined screw capped tubes with 5 ml 90% acetone using a stainless steel spatula for filter grinding. The tubes were chilled in a beaker of ice and sonicated for 5 min in an ultrasonic bath. Extracts were then filtered through 25 mm diameter syringe filters (MFS HP020, 25 mm, and 0.20 µm pore size, hydrophilic PTFE) to remove cell

and filter debris. An aliquot (0.5 ml) of acetone extract was mixed with 0.2 ml of water, and 200 µl were immediately injected. This procedure avoids peak distortion of early eluting peaks (Zapata & Garrido 1991) and prevents the loss of non-polar pigments prior to injection (Latasa et al. 2001).

HPLC analysis

Pigments were separated using a Waters Alliance HPLC System (Waters Corporation) consisting of a 2695 separations module, a Waters 996 diode-array detector (1.2 nm optical resolution) and a Waters 474 scanning fluorescence detector. Pigment separation was performed using the HPLC method of Zapata et al. (2000), with a reformulated mobile phase A. The column was a C_8 Waters Symmetry (150 × 4.6 mm, 3.5 µm particle size, 100 Å pore size). Eluent A was methanol:acetonitrile:0.025 M aqueous pyridine (50:25:25 by vol.). Eluent B was methanol:acetonitrile:acetone (20:60:20 by vol.). The elution gradient was as follows: (time (min): %B) t_0: 0%, t_{22}: 40%, t_{28}: 95%, t_{37}: 95%, t_{40}: 0%. Flow rate was 1.0 ml min^{-1}, and column temperature was fixed at 25°C using a Peltier-column thermostat (Jet-Stream Plus). Solvents were HPLC grade (Romil-*SpS*™), pyridine was reagent grade (Merck). CSIRO strains were analysed following the procedure described by Zapata et al. (2004).

Pigment identification

Pigments were identified either by co-chromatography with authentic standards obtained from SCOR reference cultures (Jeffrey et al. 1997) and diode-array spectroscopy (see Zapata et al. 2000). After checking for peak purity, spectral information was compared with a library of chlorophyll and carotenoid spectra from pigments prepared from phytoplankton cultures. Compounds such as 4-keto-19′-hexanoyloxyfucoxanthin and chl c_1-like *Kryptoperidinium*-type were isolated as described previously (Garrido & Zapata 1998, Egeland et al. 2000, Zapata et al. 2006). Novel carotenoids from *Karenia mikimotoi* were tentatively identified (Garrido et al. unpubl.). Pigment nomenclature and abbreviations were as suggested by SCOR WG 78 (Jeffrey & Mantoura 1997). For monogalactosyl-diacylglycerol (MGDG)-chl *c*-like pigments whose molecular structures have been elucidated (Garrido et al. 2000), the nomenclature was MGDG-chl c_2-*Chrysochromulina polylepis*-type (Zapata et al. 2001). For chlorophylls whose molecular structure is unknown, the pigment name includes a reference to the most likely chl *c* chromophore (chl c_1- or c_2-like), and a mention of the species in which the pigment was initially detected (e.g. chl c_2-like *Pavlova gyrans*-type, chl c_1-like *Exanthemachrysis*-type). For tentative identification of unknown pigments, the chromatographic behaviour was studied using 2 HPLC systems: the polymeric C_{18} method of Garrido & Zapata (1997) and the C_8 method of Zapata et al. (2000) were compared.

Pigment quantification

HPLC calibration by external standards was performed using chlorophyll and carotenoid standards isolated from microalgal cultures (see Zapata et al. 2000), as well as pigments supplied by DHI (Denmark). The molar extinction coefficients (ε; l mol^{-1} cm^{-1}) provided by Jeffrey (1997) were used for pigment quantification. For chl *c*-like pigments whose molar extinction coefficients are not available (i.e. chl c_3, chl c_1-like *Exanthemachrysis*-type and chl c_2-like *Pavlova gyrans*-type) the mean of the extinction coefficients for chl c_1 and c_2 at the blue absorption band (see Jeffrey et al. 1997) was used. The MGDG-chl c_2 were quantified by using the molar extinction coefficient of the chl c_2 chromophore. For fucoxanthin-related compounds (i.e. acyloxy and keto derivatives), the molar extinction coefficient for fucoxanthin was used, following the recommendations of Jeffrey et al. (1997), even though the absorption spectra of fucoxanthin-derivatives differ slightly from those of the parent compounds. Thus pigment to chl *a* ratios are expressed on a molar base (mol mol^{-1}).

RESULTS

Chromatographic resolution and pigment identities

The peak number of the pigments together with the retention time and their visible absorption maxima in eluent is shown in Table 1. Of the 63 pigments, 44 were well known chlorophylls and carotenoids previously compiled by Jeffrey et al. (1997). Structures of chl *c* pigments not included in the above review may be found in Helfrich et al. (1999) and Zapata et al. (2006), and structures of algal carotenoids in Bjørnland et al. (2000) and Egeland et al. (2000). The rest of pigments were unknown chl *c*-like compounds and carotenoids present in trace amounts.

Table 1. Elution order and visible absorption characteristics of pigments in eluent from dinophyte cultures. Wavelengths given in parentheses denote shoulders. Occurrence across the 6 pigment-based chloroplast types is indicated. MGDG: monogalactosyl-diacylglycerol

Peak no.	Pigment	Chloroplast type	Abbreviation	Time (min)	λ maxima in eluent (nm)		
1	Peridininol	1	Perid-ol	5.66		477	
2	Unknown carotenoid λ_{max} 465 nm	1	Unk-car465	7.28		465	
3	Chlorophyll c_2-like *Pavlova gyrans*-type	4	Chl c_2-like *Pg*	7.69	457	586	634
4	Chlorophyll c_3	2, 3	Chl c_3	8.02	458	591	(629)
5	Chlorophyll c_1-like *Exanthemachrysis*-type	4	Chl c_1-like *Eg*	8.31	453	585	635
6	Unknown chlorophyll c_2-like	1	Chl c_2-like 450	8.86	450	583	631
7	Chlorophyllide *a*	1–6	Chlide *a*	10.47	430	619	663
8	Divinyl protochlorophyllide *a*	1–6	MgDVP	10.83	440	574	628
9	Chlorophyll c_2	1–5	Chl c_2	11.75	453	585	634
10	Chlorophyll c_1	1, 4	Chl c_1	12.34	448	583	632
11	Peridinin	1	Peri	13.85		475	
12	Peridinin-like	1	Peri-like	14.33		478	
13	Keto-19′-butanoyloxyfucoxanthin-like	2	But-fuco-like-1	15.68		448	471
14	4-keto-19′-butanoyloxyfucoxanthin	2	But-fuco-like-2	16.29		448	470
15	Unknown carotenoid λ_{max}447	1	Unk-car447-*Cc*	16.39	(424)	447	477
16	19′-butanoyloxyfucoxanthin	2, 3	But-fuco	17.21		447	470
17	*All-trans* neoxanthin	6	*t*-neo	17.52	416	442	470
18	Heteroxanthin-like	3	Het-like*Th*	17.77	419	443	471
19	Fucoxanthin	2–4	Fuco	18.27		451	
20	9′-*cis* neoxanthin	6	Neo	19.46	413	439	466
21	Astaxanthin	1	Asta	19.58		480	
22	Keto-hexanoyloxyfucoxanthin-like	2	4k-hex-fuco-like	19.61		448	472
23	4-keto-19′-hexanoyloxyfucoxanthin	2	4k-hex-fuco	20.34		448	472
24	Violaxanthin	1, 6	Viola	20.80	415	441	470
25	Pyrrhoxanthin	1	Pyrrho	21.12		471	
26	19′-hexanoyloxyfucoxanthin	2, 3	Hex-fuco	21.22		447	471
27	Diadinochrome	1	Diadchr	22.90	(410)	430	458
28	Diadinoxanthin	1–4	Diadino	23.77	(422)	448	477
29	Dinoxanthin	1	Dino	24.93	418	442	471
30	Unknown carotenoid λ_{max} 463	4	Unk-car463-*Kf*	24.12		463	
31	*cis*-fucoxanthin	2–4	*c*-fuco	24.61		442	
32	Antheraxanthin	6	Anth	24.77	(422)	447	475
33	19′-acyloxyfucoxanthin-like	3	Acyl-fuco-like	25.26		448	471
34	Unknown carotenoid λ_{max}453	4	Unk-car453-*Kf*	25.42	(428)	453	(482)
35	Alloxanthin	5	Allo	26.06	(428)	454	483
36	Lycopene-like	1	Lyco-like-1	26.30	451	476	509
37	Diatoxanthin	1–4	Diato	26.62	(426)	453	481
38	Unknown carotenoid λ_{max}447	4	Unk-car447-*Kf*	27.10	(420)	447	(472)
39	Zeaxanthin	1–4, 6	Zea	27.45	(429)	454	480
40	Unknown carotenoid from *Lepidodinium chlorophorum*	6	Unk-car443-*Lc*	27.65	420	443	472
41	Lycopene-like	1, 2	lyco-like-2	28.00	(452)	476	507
42	Unknown carotenoid from *L. chlorophorum*	6	Unk-car443b-*Lc*	28.12	420	443	472
43	Gyroxanthin diester-like	2, 3	GyrE-like	28.58	(418)	445	472
44	Canthaxanthin	1, 3	Cantha	29.30		472	
45	Gyroxanthin diester-2	2, 3	GyrE (12:0)	29.56	(418)	445	472
46	Gyroxanthin diester-3	2, 3	GyrE (14:0)	30.46	(419)	445	471
47	Crocoxanthin	5	Croco	30.93	(431)	447	476
48	Hydroxyl echinenone	4	Hydro-echin	31.26		468	
49	β-cryptoxanthin	1	β-crypto	31.41	(426)	454	480
50	Chlorophyll *b*	6	Chl *b*	31.67	461	597	647
51	Chlorophyll c_2 MGDG from *Karlodinium armiger*	3	MGDG-chl c_2-*Ka*	32.17	454	584	634
52	Chlorophyll *a* allomer	1–6	Chl *a* allomer	32.74	420	615	662
53	Chlorophyll c_2 MGDG from *Emiliania huxleyi*	3	MGDG-chl c_2-*Eh*	32.98	454	584	634
54	Chlorophyll *a*	1–6	Chl *a*	33.29	431	617	662
55	Chlorophyll *a* epimer	1–6	Chl *a*′	33.51	431	617	662
56	Chlorophyll c_2 MGDG from *Chrysochromulina polylepis*	2, 3	MGDG-chl c_2-*Cp*	33.74	454	584	634
57	β,ψ-carotene-like	4	βψ-car-like	34.28	(437)	463	492
58	β,ψ-carotene	4	βψ-car	34.48	(437)	463	493
59	Pheophytin *a*	1–6	Pheo *a*	35.32	409	609	665
60	Chlorophyll c_2 MGDG from *Takayama* cf. *helix*	3	MGDG-chl c_2-*Th*	35.45	455	583	634
61	β,ε-carotene	2–6	βε-car	35.49	(422)	447	475
62	β,β-carotene	1–5	ββ-car	35.67	(426)	454	480
63	*Cis*-β,β-carotene	4	*c*-ββ-car	35.94	(426)	452	478

Pigment composition: chlorophylls

Thirteen chlorophylls, i.e. chl *a*, *b* and 11 chl *c*-type pigments (7 polar chl *c* and 4 non-polar chl c_2-like), were detected. Chl c_2 (peak 9) together with divinyl protochlorophyllide (MgDVP; peak 8) were always present in chl *c*-containing dinoflagellates; other chl *c* pigments showed a heterogeneous distribution, in some cases at trace levels. Chl c_1 (peak 10) was detected in several peridinin-containing dinoflagellates (e.g. *Peridinium aciculiferum*, *Gambierdiscus excentricus*), as well as in the fucoxanthin-containing dinoflagellates *Durinskia baltica* and *Kryptoperidinium foliaceum.* Chl c_3 (peak 4) was only present in the genera *Karenia*, *Karlodinium* and *Takayama* (Family Kareniaceae). The MGDG-chl c_2-*Chrysochromulina polylepis*-type (peak 56) was a minor peak in *Karenia* spp., some *Karlodinium* species (*K. armiger*, *K. decipiens*) and *T.* cf. *helix*. Two novel non-polar chl c_2-like pigments were also detected, the first (peak 51) in *K. armiger* eluted just before the MGDG-chl c_2 *Emiliania huxleyi*-type (peak 53), and the second (peak 60) in *T.* cf. *helix* at a higher retention time than the MGDG-chl c_2-*C. polylepis*-type (peak 56). Red-shifted chl c_1 (peak 5) and chl c_2 (peak 3), having absorption spectra and chromatographic behaviour similar to those of chl c_1-like from *Exanthemachrysis gayraliae* (Van Lenning et al. 2003) and chl c_2-like from *Pavlova gyrans* (Fawley 1989), occurred in *D. baltica* and *K. foliaceum*. Blue-shifted chl c_2-like pigment (peak 6, λ_{max} = 450 nm) was detected as traces in several peridinin-containing dinoflagellates. Chl *b* (peak 50) was restricted to *Lepidodinium* (PT) *chlorophorum.*

Carotenoid composition

Among the 47 carotenoids detected in the dinoflagellates studied (see Table 1), 31 were well-known compounds and 16 were unknown compounds (most of them occurring at minor or trace levels, probably optical or geometrical isomers of known carotenoids). Results from electron visible absorption and chromatographic behaviour in 2 HPLC systems with distinct selectivity, i.e. the C_8 HPLC and the polymeric C_{18} method of Garrido & Zapata (1997), were taken into account for tentative identification (data not shown).

Peridininol (peak 1), the deacetylated derivative of peridinin (peak 11), was the most polar carotenoid detected. It co-occurred with peridinin as well as a minor peridinin-like pigment (peak 12) eluting just after peridinin. Diadinochrome (peak 27), the 5,8-epoxide rearrangement of diadinoxanthin, diadinoxanthin (peak 28), dinoxanthin (peak 29) and diatoxanthin (peak 37) completed the major carotenoids detected in peridinin-containing dinoflagellates. Other carotenoids that were less abundant or restricted to a few species were: a diadinoxanthin-like pigment (peak 15) detected in *Coolia canariensis* (VGO775), astaxanthin (peak 21), violaxanthin (peak 24), pyrrhoxanthin (peak 25), *trans*-neoxanthin (peak 17) and zeaxanthin (peak 39).

Carotenoids of the genus *Karenia* included 3 novel compounds: peaks 13, 14 and 22. The visible absorption spectra of these carotenoids isolated from *K. mikimotoi* (CCMP429) are shown in Fig. 1; for comparative purposes, visible spectra of 19′-butanoyloxyfucoxanthin (peak 16), 19′-hexanoyloxyfucoxanthin (peak 26) and 4-keto-19′-hexanoyloxyfucoxanthin (peak 23) are also depicted. A major gyroxanthin diester carotenoid (peak 45) occurred in *Karenia* and *Karlodinium* species; in addition, a second compound (peak 46) with a similar absorption spectrum was detected in *Karlodinium veneficum* strains at variable proportions. A third gyroxanthin diester-like pigment (peak 43), less retained than the previous ones, as well as less abundant, was detected in *K. mikimotoi*.

Alloxanthin (peak 35) and crocoxanthin (peak 47) were detected in *Dinophysis acuminata*, *D. acuta*, *D. caudata* and *D. tripos*; monadoxanthin was absent. Carotenoids of the chl *b*-containing dinoflagellate *Lepidodinium chlorophorum* included both forms of neoxanthin (*all-trans* neoxanthin [peak 17] and 9′-*cis* neoxanthin [peak 20]), violaxanthin (peak 24), antheraxanthin (peak 32), zeaxanthin (peak 39) and a major unknown carotenoid (peak 40).

Pigment-based chloroplast types in dinoflagellates

According to the pigment composition of the dinoflagellates analysed, 6 pigment-based chloroplast types (hereafter 'chloroplast types') were defined, and representative chromatograms are shown in Fig. 2. Chlorophyll and carotenoid composition of the 6 chloroplast types are summarised in Table 2.

Most dinoflagellates species were peridinin-containing organisms labeled as chloroplast Type 1 (71% of species, 51% of strains), with chl c_2 (peak 9) as the major accessory chlorophyll and traces of MgDVP (peak 8). Chl c_1 (peak 10) was present in some cases at lower concentrations than chl c_2 (e.g. *Gambierdiscus excentricus* VGO790, *Peridinium aci-*

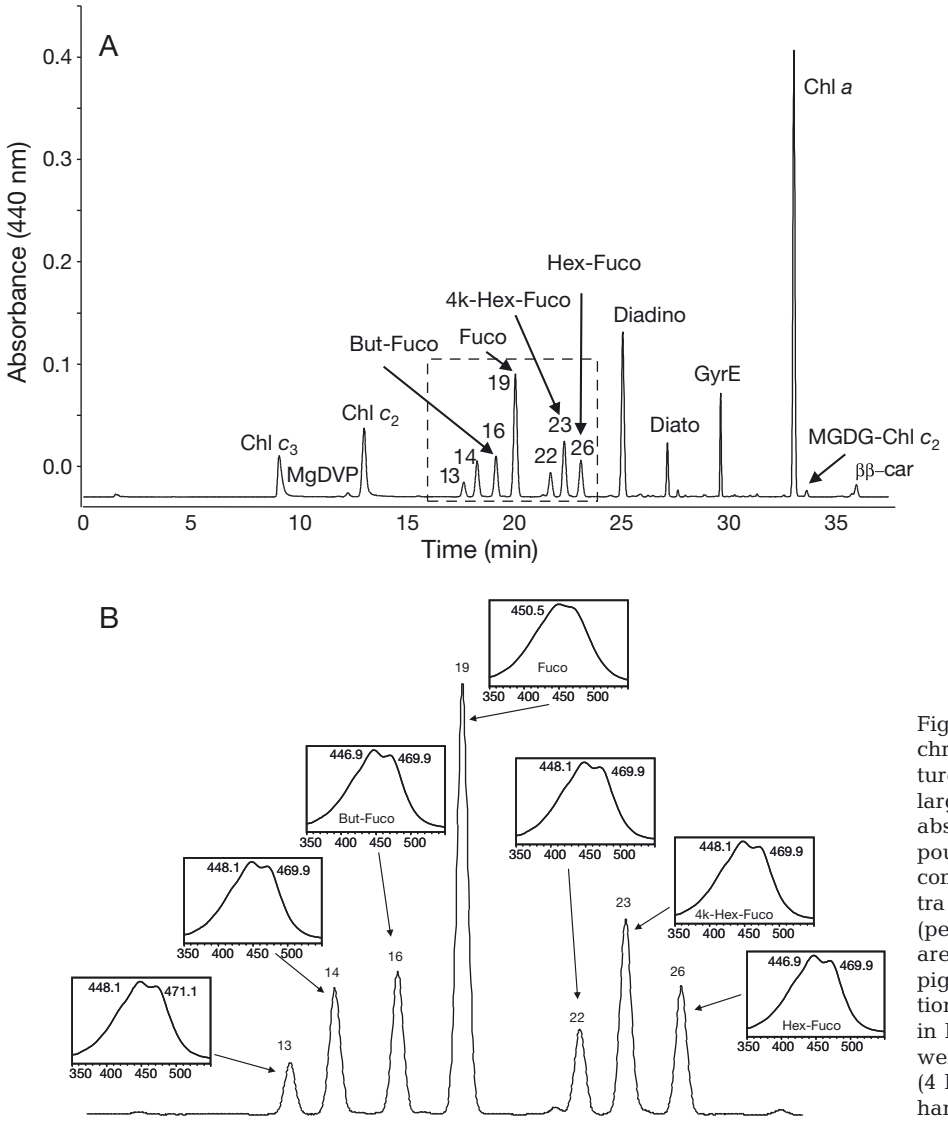

Fig. 1. *Karenia mikimotoi.* (A) HPLC chromatogram from a massive culture of strain CCMP429. (B) Enlarged section showing the visible absorption spectra of 3 novel compounds (peaks 13, 14 and 22). For comparative purposes, visible spectra of But-fuco (peak 16), Hex-fuco (peak 26) and 4k-Hex-fuco (peak 23) are also depicted (see Table 1 for full pigment names). Pigment proportions cannot be compared with those in Fig. 2 because growth conditions were different in massive cultures (4 l) due to self-shading and longer harvesting periods (10 to 15 d)

culiferum PAER-2, *Protoceratium reticulatum* CCMP1720), although chl c_1 was the major chl *c* pigment in *Gyrodinium uncatenum* CS-289/3. The carotenoids peridinin (peak 11) and dinoxanthin (peak 29) were specific to chloroplast Type 1 dinoflagellates. Other carotenoids were diadinoxanthin (peak 28), diadinochrome (peak 27), diatoxanthin (peak 37) and ββ-car (peak 62). Overall, 11 genera, including 44 species (83 strains) of the dinoflagellates studied, belonged to chloroplast Type 1.

Chloroplast Type 2 grouped dinoflagellates with Fuco (peak 19), 19′-acyloxyfucoxanthins and their keto derivatives (up to 6 fucoxanthin-related compounds: peaks 13, 14, 16, 22, 23 and 26), and gyroxanthin diesters (up to 3 compounds: peaks 43, 45 and 46). The array of chl *c* included: chl c_2 (peak 9), chl c_3 (peak 4), MgDVP (peak 8) and MGDG-chl c_2-*Chrysochromulina polylepis*-type (peak 56). Only the genus *Karenia* (5 species, 8 strains) belonged to chloroplast Type 2. Fucoxanthin (peak 19) was the major carotenoid in *K. brevis*, *K. mikimotoi* and *K. selliformis*, but Hex-fuco (peak 26) was the most abundant carotenoid in *K. papilonacea* and *K. umbella*. Different forms of gyroxanthin diester were tentatively identified: $C_{12:0}$ (peak 45), the major form described by Bjørnland et al. (2003), and 2 minor components tentatively assigned as $C_{14:0}$ (peak 46), and a less retained gyroxanthin diester-like pigment (peak 43). The fingerprint of chloroplast Type 2 dinoflagellates with six 19′-acyloxyderivatives is unique for the group of 5 *Karenia* species (8 strains) analysed.

Chloroplast Type 3 resembled Type 2, but it lacked the diverse fucoxanthin pool observed in *Karenia* species. But-fuco and Hex-fuco were the major acyl-

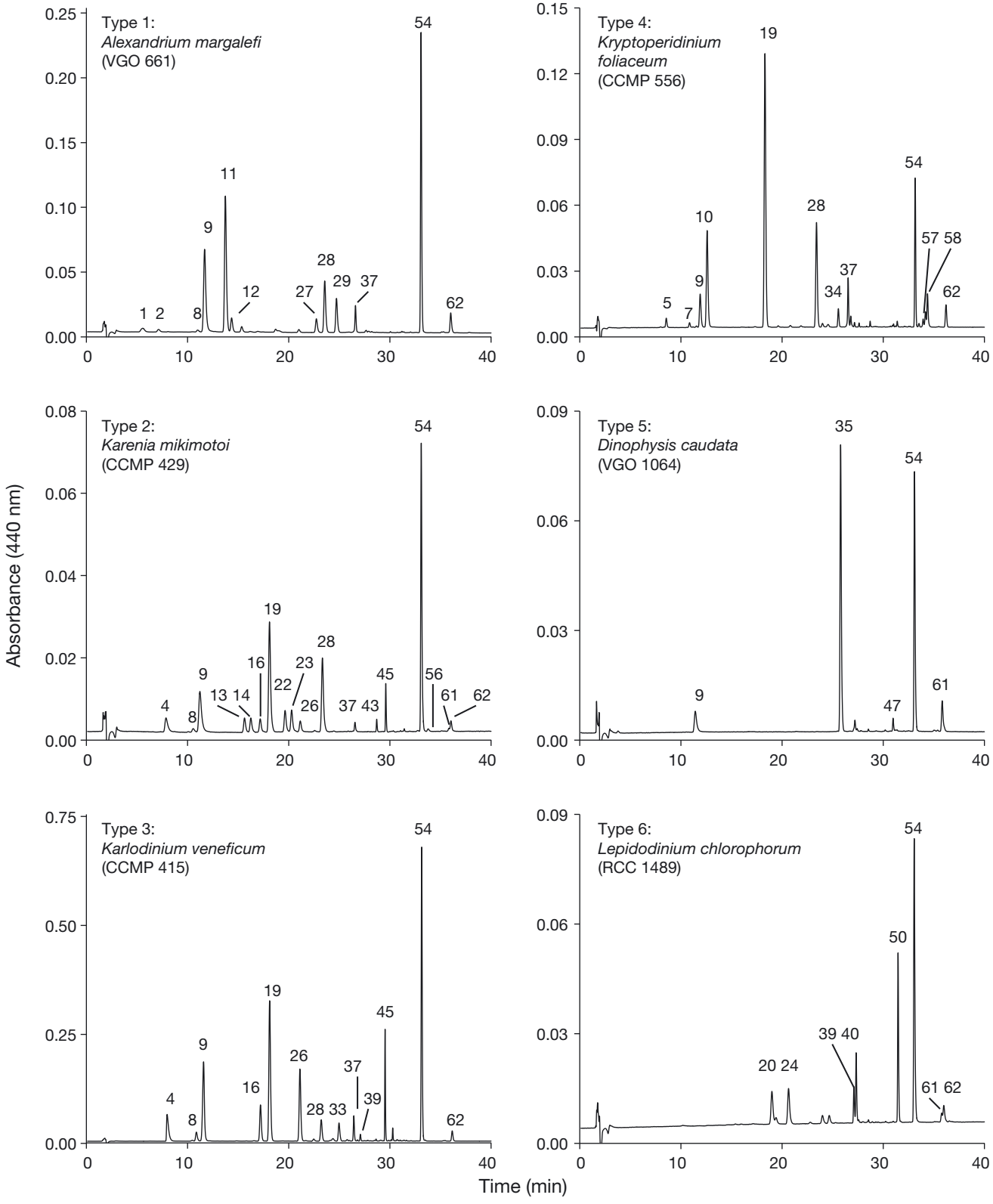

Fig. 2. HPLC chromatograms of dinoflagellates belonging to major chloroplast types. Type 1: *Alexandrium margalefi* (VGO661); Type 2: *Karenia mikimotoi* (CCMP429); Type 3: *Karlodinium veneficum* (CCMP415); Type 4: *Kryptoperidinium foliaceum* (VGO556); Type 5: *Dinophysis caudata* (VGO1064); Type 6: *Lepidodinium chlorophorum* (RCC1489). Detection by absorbance at 440 nm. Peak identifications as in Table 1

Table 2. Distribution of chlorophylls (chl) and carotenoids among pigment-based chloroplast types in Dinophyta. ●: consistent occurrence; ○: occasional occurrence. Abbreviations as in Table 1

Pigment	Pigment-based chloroplast type					
	1	2	3	4	5	6
Chlorophylls	Chl *c*					Chl *b*
Chl c_2-like *Pg*	–	–	–	●	–	–
Chl c_1-like *Eg*	–	–	–	●	–	
Chl c_3	–	●	●	–	–	–
MgDVP	●	●	●	●	●	●
Chl c_2	●	●	●	●	●	–
Chl c_1	○	–	–	●	–	–
Chl *b*	–	–	–	–	–	●
Chl *a*	●	●	●	●	●	●
MGDG-chl c_2	–	●	○	–	–	–
Carotenoids	Peri-type	Fuco-type			Allo-type	Vio/Neo-type
Peridininol	●	–	–	–	–	–
Unk 1/Unk 2	●	–	–	–	–	–
Peridinin	●	–	–	–	–	–
cis-Peridinin	●	–	–	–	–	–
Keto-19′-but-fuco-like	–	●	–	–	–	–
4-keto-19′-but-fuco	–	●	–	–	–	–
19′-but-fuco	–	●	●	–	–	–
Fucoxanthin	–	●	●	●	–	–
Keto-19′-hex-fuco-like	–	●	–	–	–	–
4-keto-19′-hex-fuco	–	●	○	–	–	–
19′-hex-fuco	–	●	●	–	–	–
Violaxanthin	–	–	–	○	–	●
Diadinochrome	●	–	–	–	–	–
Diadinoxanthin	●	●	●	●	●	–
Dinoxanthin	●	–	–	–	–	–
Alloxanthin	–	–	–	–	●	–
Diatoxanthin	○	○	○	○	○	–
Zeaxanthin	○	○	○	○	–	○
Unknown carotenoid *Lc*	–	–	–	–	–	●
Lycopene-like	○	○	○	○	–	○
Gyroxanthin diester 1	–	●	●	–	–	–
Gyroxanthin diester 2	–	●	●	–	–	–
Gyroxanthin diester 3	–	●	●	–	–	–
β,ψ-carotene	–	–	–	●	–	●
β,ε-carotene	–	●	●	●	●	●
β,β-carotene	●	●	●	●	○	●

oxyderivatives detected in *Karlodinium* spp. with 19′-acyloxyfucoxanthin-like (peak 33) at trace levels (Acyl-fuco-like:chl *a* = 0.02) and no keto-19′-acyloxy-derivatives. The major form of gyroxanthin diester was the $C_{12:0}$ acyl ester at C-19 (peak 45), but $C_{14:0}$ (peak 46) was also abundant in *Karlodinium armiger*, *K. decipiens* and *K. veneficum*. However, *Takayama* cf. *helix* (VGO341), which was also included in chloroplast Type-3, lacked gyroxanthin diesters. Two genera, with 4 species (11 strains), of the dinoflagellates studied belonged to chloroplast Type 3.

Chloroplast Type 4, comprising fucoxanthin-containing dinoflagellates with no acyloxyderivatives, included 2 species from different genera: *Durinskia baltica* (1 strain) and *Kryptoperidinium foliaceum* (2 strains). A characteristic feature is the occurrence of β,ψ-carotene (peak 58). Chl c_1 (peak 10) was the dominant chl *c*-type pigment, with chl c_2 (peak 9) second in importance. Two minor chl *c*, viz. chl c_1-like *Exanthemachrysis gayraelae*-type (peak 5) and chl c_2-like *Pavlova gyrans*-type (peak 3), were also detected.

Chloroplast Type 5 included dinoflagellates from the genus *Dinophysis* (4 species and 4 strains) with cryptophyte-like chloroplasts. Acetylenic alloxanthin (peak 35) was the major carotenoid, together with crocoxanthin (peak 47), and chl c_2 (peak 9) and MgDVP (peak 8) as the accessory chlorophylls.

Chloroplast Type 6 has green algae-like chloroplast pigments: i.e. the accessory chl *b* (peak 50), 9′-*cis* neoxanthin (peak 20), *all-trans* neoxanthin isomer (peak 17) and violaxanthin (peak 24). Neither lutein nor free-loroxanthin, siphonaxanthin or its esters were detected. The major carotenoid was an unknown compound (peak 40, λ_{max}: 420, 443, 475 nm; band ratio (%III/II) = 87) eluting after zeaxanthin (peak 39, see Table 1). Using another HPLC method to improve carotenoid separation in *Tetraselmis* species (Garrido et al. 2009), peak 40 eluted before a lutein standard (data not shown). The 4 strains of *Lepidodinium chlorophorum* analysed (BAHME100, RCC1488, RCC1489 and Dino16EUH) showed a similar carotenoid profile.

Quantitative pigment data: chlorophyll pigments

The abundance of chlorophyll and carotenoid pigments expressed as molar ratios with respect to chl *a* are shown in Tables 3 to 5 (corresponding mass [w:w] ratios are shown in Tables S2 to S4). Chl c_2 was the most abundant chl *c* pigment in chloroplast Types 1, 2, 3 and 5. The chl c_2 to chl *a* ratio in peridinin-containing dinoflagellates (Table 3) ranged from 0.03 (*Prorocentrum levis*) to 0.57 (*Amphidinium carterae*). The occurrence of MgDVP in trace amounts was a general trait in chl *c*-containing species (ratios not shown in tables). Chl c_1 occurred in several peridinin-

containing dinoflagellates (Type 1), usually in traces. Intermediate values were observed in *Gambierdiscus excentricus* (chl c_1:chl *a* = 0.06) and *Peridinium aciculiferum* (0.16), and the highest in *Gyrodinium uncatenum* (chl c_1:chl *a* = 0.41), where chl c_1 was the major chl *c* pigment (chl c_1:c_2 = 1.46). Chloroplast Types 2 and 3 (Table 4) showed chl c_2 and c_3 with chl c_3:chl c_2 ratios varying from 0.29 to 0.58. Chloroplast Type 4 (Table 5) contained higher values of chl c_1 than chl c_2 (chl c_1:chl c_2 = 1.38–2.25). Chl c_1-like *Exanthemachrysis*-type and chl c_2-like *Pavlova*-type were minor pigments in *Kryptoperidinium foliaceum* and *Durinskia baltica*, respectively, representing ~6% of the chl *c* pool. MGDG-chl c_2 *Chrysochromulina polylepis*-type (peak 56) occurred as a minor pigment in Type 2 (MGDG-chl c_2-*C. polylepis*-type:chl *a* ≤ 0.005), and certain *Karlodinium* species (e.g. *K. armiger* and *K. decipiens*) were included in chloroplast Type 3.

Finally, chl *b*:chl *a* ratios (Type 6, Table 5) varied among the *Lepidodinium chlorophorum* strains. The ratio was low (0.08) for the North Sea strain (BAHME 100), while the Nervion River isolate (Dino16EUH) showed the highest chl *b*:chl *a* ratio (0.74).

Quantitative pigment data: carotenoids

Peridinin was the major carotenoid in almost all peridinin-containing dinoflagellates (Table 3). Only in a few cases did the Diadino:chl *a* ratio surpass Peri:chl *a* ratios, reflecting a high xanthophyll cycle activity. The Peri:chl *a* ratio (Table 3) ranged from 0.54 in *Barrufeta bravensis* (VGO864) to 2.06 in *Coolia canariensis* VGO787. If normalised to chl c_2, the lower values correspond to Gymnodiniales (Peri:chl c_2 = 1.60–2.91) and Peridiniales (Peri:chl c_2 = 1.39–4.87). Prorocentrales showed higher ratios (Peri:chl c_2 = 3.51–38.92), especially the benthic, symmetric species of *Prorocentrum*. Diadinoxanthin was usually the second major carotenoid, with ratios of Diadino:chl *a* ranging from 0.28, both in *Alexandrium pseudogonyaulax* VGO706 and in *A. catenella* AL96, to 1.09 in *P. lima* PL2V. Dinoxanthin:chl *a* ratios were rather constant in most strains (mean ± SD = 0.18 ± 0.07), although the ratio ranged from 0.01 (*Neoceratium furca* Nfurca1) to 0.42 (*B. bravensis* VGO860).

In chloroplast Type 2 (Table 4), Fuco was the major carotenoid in *Karenia brevis*, *K. mikimotoi* and *K. selliformis* (Fuco:chl *a* = 0.24–0.34) whereas Hex-fuco was dominant in *K. papilonacea* (Hex-fuco:chl *a* = 0.29) and *K. umbella* (Hex-fuco:chl *a* = 0. 32; Table 4). The contribution of acyloxyfucoxanthins (6 compounds) to the fucoxanthin total pool varied among strains. The Hex-fuco derivatives were always more abundant than the But-fuco pigments. The fucoxanthin pool in chloroplast Type 3 was formed by Fuco, But-fuco and Hex-fuco with no keto-derivatives. Fuco was the major carotenoid (Table 4) in 5 out of 6 *Karlodinium veneficum* strains (Fuco:chl *a* = 0.30–0.75), and Hex-fuco was the major carotenoid in *K. armiger*, *K. decipiens* and *K. veneficum* VGO870 (Hex-Fuco:chl *a* = 0.21–0.45). *Takayama* cf. *helix* showed Fuco as major carotenoid (Fuco:chl *a* = 1.18), with only a minor contribution of Hex-fuco (Hex-fuco:chl *a* = 0.06).

Gyroxanthin diester pigments were present in chloroplast Types 2 and 3 except in *Takayama* cf. *helix* (Table 4). In *Karenia* species, the pigment ratio of the major form (peak 45: GyrE $C_{12:0}$) was rather constant (GyrE:chl *a* = 0.11–0.17); a more polar gyroxanthin-like compound (peak 43) was the second most abundant form (GyrE1-like:chl *a* = 0.01–0.03). In *Karlodinium* species, GyrE $C_{12:0}$ (peak 45) was also the major compound (GyrE:chl *a* = 0.11–0.24), with variable contribution of the more retained GyrE $C_{14:0}$ (peak 46, GyrE:chl *a* = 0.02–0.09).

Fucoxanthin was the major carotenoid in chloroplast Type 4 (0.52 to 0.75), while β,ψ-carotene could only be quantified in *Kryptoperidinium foliaceum* VGO556 (0.052). Pigment ratios in chloroplast Type 5 (*Dinophysis* spp.) were rather constant with Allo:chl *a* = 1.32–1.62, Croco:chl *a* = 0.05–0.08 and βε-car:chl *a* = 0.15–0.20.

Chloroplast Type 6, represented by 4 strains of *Lepidodinium chlorophorum*, showed marked differences in carotenoids and chl *b* pigment ratios (Table 5). The North Sea strain (BAH100ME, the type culture) showed violaxanthin as the major carotenoid (Viola:chl *a* = 0.32), with an unknown carotenoid (peak 46) as the second most important carotenoid (Unk car:chl *a* = 0.18). In both British Channel (RCC1488, RCC1489) and Nervion River (Dino16 EUH) strains, the unknown carotenoid was the major carotenoid (Unk car:chl *a* = 0.14–0.17), followed by violaxanthin (Viola:chl *a* = 0.08–0.32). Lutein was detected as traces in the 4 strains. βε-car was the major carotene in BAH100ME and Dino16EUH strains (βε-car:chl*a* = 0.03–0.11).

Occurrence of pigment-based chloroplast types across dinoflagellate taxa

The distribution patterns of pigment-based chloroplast types across dinoflagellate taxa are summarised in Table 6. These examples showed the different

Table 3. Molar pigment ratios to chlorophyll (chl) *a* and their variability in chloroplast Type 1

Order and species	Strain code	Peri:chl c_2	Peri:chl *a*	Chl c_2:chl *a*	Chl c_1:chl *a*	Diadino:chl *a*	Dino:chl *a*
Gonyaulacales							
Alexandrium affine	PA2V	4.08	1.08	0.27	0.00	0.52	0.22
A. andersonii	CCMP1718	3.36	1.14	0.34	0.00	0.60	0.17
A. andersonii	VGO664	4.48	1.39	0.31	0.00	0.64	0.09
A. andersonii	SZN-12	3.19	0.61	0.19	0.00	0.41	0.18
A. catenella	AT02	3.05	0.85	0.28	0.00	0.63	0.24
A. catenella	VGO609	3.00	0.84	0.28	0.00	0.43	0.39
A. catenella	AL96	2.90	0.64	0.22	0.00	0.28	0.14
A. insuetum	ICMB218	3.16	1.02	0.32	0.00	0.38	0.11
A. margalefii	ICMB	3.47	1.11	0.32	0.00	0.41	0.14
A. margalefii	VGO661	2.94	0.90	0.31	0.00	0.30	0.15
A.minutum	AL1V	5.05	1.34	0.27	0.00	0.40	0.17
A. minutum	AMAD01	3.31	1.36	0.41	0.00	0.46	0.17
A. minutum	CLONE4	2.68	1.07	0.40	0.00	0.43	0.20
A. minutum	VGO577	3.14	1.11	0.35	0.00	0.47	0.16
A. ostenfeldii	AOTV-A1A	3.29	0.98	0.30	0.02	0.84	0.20
A. ostenfeldii	AOTV-A4A	3.04	0.89	0.29	0.01	0.80	0.16
A. peruvianum	AM10C	4.94	1.24	0.25	0.00	0.63	0.14
A. pseudogonyaulax	VGO706	3.41	1.28	0.38	0.00	0.28	0.07
A. tamarense	CCMP1493	3.24	0.95	0.29	0.00	0.40	0.17
A. tamarense	MDQ1096	3.30	1.14	0.34	0.00	0.50	0.18
A. tamarense	PE1V	2.81	0.95	0.34	0.00	0.38	0.16
A. tamarense	VGO553	3.59	1.11	0.31	0.00	0.44	0.16
A. tamutum	VGO617	3.30	1.21	0.37	0.00	0.59	0.17
A. taylori	AM8V	2.16	0.55	0.27	0.00	0.67	0.25
A. taylori	VGO703	2.36	0.63	0.27	0.00	0.37	0.23
Coolia canariensis	VGO775	4.11	1.56	0.38	0.00	0.63	0.15
C. canariensis	VGO787	4.00	2.06	0.52	0.00	0.57	0.13
C. monotis	CM2V	3.14	1.02	0.33	0.00	0.58	0.16
C. monotis	CM6V	3.01	1.11	0.37	0.00	0.65	0.17
C. monotis	RIKZ4	2.49	1.08	0.43	0.00	0.59	0.18
C. monotis	CCMP1345	2.65	1.15	0.43	0.00	0.47	0.19
Coolia sp.	VGO923	3.00	1.05	0.35	0.00	0.42	0.13
C. tropicalis	CCMP1744	2.23	1.14	0.52	0.07	0.60	0.17
Fragilidium sp.	VGO692	3.05	0.96	0.31	0.00	0.68	0.19
Fragilidium sp.	IO 91-01	2.84	1.03	0.36	0.00	0.52	0.20
Gambierdiscus excentricus	VGO790	3.39	1.52	0.45	0.06	0.62	0.20
Gambierdiscus sp.	VGO920	2.72	0.91	0.33	0.04	0.53	0.18
Gambierdiscus sp.	KC81G1	2.72	1.10	0.41	0.07	0.62	0.16
Lingulodinium polyedrum	LP4V	3.85	1.11	0.29	0.00	0.37	0.20
L. polyedrium	LP9V	4.12	1.10	0.27	0.00	0.37	0.19
Neoceratium furca	Nfurca1	2.81	0.72	0.26	0.00	0.38	0.01
Ostreopsis ovata	OS01BR	3.00	1.19	0.40	0.00	0.76	0.14
O. cf. *ovata*	VGO611	2.95	1.18	0.40	0.00	0.47	0.13
O. cf. *siamensis*	OS3V	3.42	1.43	0.42	0.00	1.01	0.12
O. cf. *siamensis*	VGO613	3.49	1.37	0.39	0.00	0.74	0.14
O. cf. *siamensis*	VGO883	3.35	1.22	0.37	0.00	0.60	0.14
Protoceratium reticulatum	GG1AM	2.84	1.02	0.36	0.01	0.40	0.16
P. reticulatum	CCMP404	3.88	0.94	0.24	0.04	0.62	0.19
P. reticulatum	CCMP1720	4.26	1.02	0.24	0.03	0.64	0.15
Gymnodiniales							
Akashiwo sanguinea	VGO138	2.20	0.68	0.31	0.00	0.68	0.20
A. sanguinea	VGO626	2.91	0.75	0.25	0.00	0.41	0.20
Amphidinium carterae	A01BR	2.04	1.17	0.57	0.00	0.90	0.10
A. carterae	ACMK03	1.60	0.87	0.54	0.00	0.77	0.19
A. carterae	ACRN02	1.81	1.09	0.60	0.00	0.70	0.07
A. cf. *carterae*	A1V	2.29	0.94	0.41	0.00	0.56	0.09
Barrufeta bravensis	VGO859	1.85	0.61	0.34	0.00	0.75	0.18
B. bravensis	VGO860	1.93	0.79	0.41	0.00	0.81	0.42
B. bravensis	VGO864	1.92	0.54	0.29	0.00	0.89	0.32
Gymnodinium catenatum	GC11V	2.40	0.72	0.30	0.00	0.62	0.28

Table 3 (continued)

Order and species	Strain code	Peri:chl c_2	Peri:chl *a*	Chl c_2:chl *a*	Chl c_1:chl *a*	Diadino:chl *a*	Dino:chl *a*
G. catenatum	GC31AM	1.93	0.58	0.30	0.00	0.51	0.28
G. catenatum	CS-302	1.84	0.82	0.44	0.00	0.64	0.24
G. impudicum	GY1VA	1.94	0.66	0.35	0.00	0.59	0.29
G. instriatum	VGO642	2.51	0.84	0.34	0.00	0.73	0.15
G. cf. *microreticulatum*	VGO581	1.93	0.59	0.30	0.02	0.55	0.20
G. nolleri	DK5	2.89	1.02	0.35	0.00	0.42	0.32
Gyrodinium uncatenum	CS289-3	2.54	0.71	0.28	0.41	0.75	0.12
Peridiniales							
Heterocapsa niei	VGO399	1.39	0.79	0.56	0.12	0.52	0.18
H. triquetra	VGO1053	1.40	0.70	0.50	0.00	0.73	0.16
Peridinium aciculiferum	PAER-1	2.62	0.81	0.31	0.14	0.43	0.10
P. aciculiferum	PAER-2	2.75	0.88	0.32	0.16	0.44	0.10
Scrippsiella hangoei	STHV-1	1.95	0.89	0.45	0.02	0.51	0.11
S. hangoei	STHV-2	1.94	0.92	0.46	0.02	0.53	0.10
S. hangoei	STHV-5	2.04	0.81	0.38	0.02	0.44	0.09
S. hangoei	STHV-6	1.96	0.85	0.43	0.03	0.52	0.11
Scrippsiella sp.	S3V	4.87	1.18	0.24	0.00	0.54	0.10
Prorocentrales							
Prorocentrum arenarium	VGO776	7.17	0.95	0.13	0.03	0.59	0.27
P. belizeanum	PBMA01	19.11	1.65	0.09	0.00	0.80	0.25
P. belizeanum	VGO867	24.54	1.43	0.06	0.01	0.75	0.22
P. compressum	VGO621	4.59	1.17	0.26	0.00	0.45	0.12
P. cf. *faustiae*	VGO894	7.13	1.16	0.16	0.00	0.50	0.26
P. levis	VGO777	38.92	1.20	0.03	0.01	0.62	0.24
P. levis	VGO957	15.04	0.97	0.07	0.00	0.61	0.30
P. lima	PL2V	11.66	1.54	0.13	0.00	1.09	0.30
P. cf. *lima*	VGO620	3.68	1.32	0.36	0.01	0.82	0.30
P. micans	PM1V	7.66	1.24	0.16	0.00	0.62	0.13
P. minimum	VGO365	4.59	1.21	0.27	0.00	0.53	0.07
P. minimum	VGO367	10.31	1.65	0.16	0.00	0.43	0.08
P. nux	UTEX1008	3.51	1.51	0.43	0.00	0.73	0.11
P. rathymum	VGO893	9.94	1.59	0.16	0.00	0.64	0.16
P. rostratum	PR1V	18.00	1.69	0.09	0.00	0.46	0.12
P. triestinum	PT2V	4.13	1.35	0.33	0.00	0.64	0.14
Thoracosphaerales							
Thoracosphaera heimii	CCMP1069	2.35	1.13	0.48	0.00	0.89	0.05
Mean		4.57	1.07	0.33	0.01	0.57	0.18
SD		5.35	0.29	0.12	0.02	0.16	0.07
N		90	90	90	21	90	90

specificity of the chloroplast types across Dinophyta. All orders, except Dinophysiales, include some genera with chloroplast Type 1 chloroplast. Besides representatives of Type 1, Gymnodiniales include other genera of chloroplast Types 2, 3 and 6. Finally, the order Peridiniales encompasses representatives of pigment Type 4 as well as the already mentioned Type 1.

Three chloroplast types contain a single genus (Type 2 associated with *Karenia*, Type 5 with *Dinophysis* and Type 6 with *Lepidodinium*), while Type 3 is linked to 2 genera (*Karlodinium* and *Takayama*), and Type 4 is confined to 3 genera (*Durinskia*, *Galeidinium* and *Kryptoperidinium*).

DISCUSSION

Comparison with previous surveys of Dinophyta

The first comprehensive study of chloroplast pigments in dinoflagellates used thin layer chromatography to describe the chlorophyll and carotenoid composition of 22 species belonging to the orders Gymnodiniales, Gonyaulacales, Peridiniales and Prorocentrales (Jeffrey et al. 1975). Peridinin was the major carotenoid in 19 of the species, while fucoxanthin was the major carotenoid in 3 peridinin-lacking Peridiniales. Since then, dinoflagel-

Table 4. Molar pigment ratios (accessory chl:chl *a*) in chloroplast Types 2 and 3. MGDG-chl *c*:monogalactosyl-diacylglycerol-chl *c* compounds. (–): pigments not found

Species and strain code	Chl c_3:chl *a*	Chl c_2:chl *a*	MGDG-chl c_2:chl *a* Peak 51	MGDG-chl c_2:chl *a* Peak 56	MGDG-chl c_2:chl *a* Peak 60	Chl c_3:chl c_2	Σchls *c*:chl *a*	But-fuco-like-1	But-fuco-like-2	But-fuco	Fuco	4k-hex-fuco-like	4k-hex-fuco	Hex-fuco	GyrE1-like	GyrE2 $C_{12:0}$	GyrE3 $C_{14:0}$
Chloroplast Type 2																	
Karenia brevis CCMP718	0.07	0.12	–	0.005	–	0.58	0.20	0.04	0.07	0.08	0.34	0.05	0.11	0.07	–	0.11	–
K. brevis CCMP2281	0.07	0.15	–	0.004	–	0.47	0.22	0.06	0.06	0.08	0.24	0.09	0.10	0.09	–	0.14	–
K. mikimotoi CCMP429	0.08	0.16	–	0.002	–	0.47	0.24	0.07	0.07	0.10	0.33	0.11	0.11	0.09	0.02	0.11	–
K. papilonacea VGO679	0.05	0.13	–	0.005	–	0.44	0.19	0.02	0.04	0.13	0.14	0.09	0.09	0.29	0.03	0.17	0.01
K. selliformis VGO875	0.08	0.16	–	0.003	–	0.51	0.24	0.08	0.09	0.08	0.27	0.10	0.12	0.08	0.01	0.12	0.01
K. umbella Gy2DE	0.07	0.16	–	0.002	–	0.58	0.23	0.01	0.01	0.13	0.27	0.10	0.08	0.32	–	0.16	–
Chloroplast Type 3																	
Karlodinium armiger GC-7	0.08	0.15	0.010	0.010	0.002	0.55	0.25	–	–	0.01	0.34	–	–	0.45	–	0.11	0.05
K. decipiens Nervión34	0.07	0.22	–	0.002	–	0.33	0.29	–	–	0.25	0.22	–	–	0.44	–	0.14	0.09
K. veneficum CCMP415	0.08	0.22	–	–	–	0.38	0.30	–	–	0.13	0.56	–	–	0.31	–	0.24	0.02
K. veneficum CCMP1974	0.08	0.22	–	–	–	0.35	0.30	–	–	0.16	0.44	–	–	0.28	–	0.16	0.09
K. veneficum CS-310	0.10	0.23	–	–	–	0.38	0.33	–	–	0.18	0.75	–	–	0.33	–	0.20	0.04
K. veneficum GC-4	0.06	0.18	–	–	–	0.29	0.30	–	–	0.08	0.72	–	–	0.21	–	0.19	0.04
K. veneficum VGO691	0.07	0.19	–	–	–	0.38	0.26	–	–	0.10	0.58	–	–	0.25	–	0.20	0.03
K. veneficum VGO870	0.07	0.21	–	–	–	0.31	0.28	–	–	0.19	0.30	–	–	0.39	–	0.20	0.02
Takayama cf. *helix* VGO341	0.07	0.21	0.005	0.013	0.007	0.33	0.31	–	–	–	1.18	–	–	0.06	–	–	–

Table 5. Molar pigment ratios to chlorophyll (chl) *a* in chloroplast Types 4, 5 and 6. tr.: trace amounts; other abbreviations as in Table 1

Chloroplast Type 4	Chl c_2	Chl c_1-like *Eg*	MgDVP	Chl c_1	Fuco	βψ-car		
Peridinium balticum CS-33	0.04	0.005	–	0.09	0.54	tr.		
Kryptoperidinium foliaceum CS-37	0.06	0.009	0.002	0.11	0.52	tr.		
K. foliaceum VGO556	0.08	0.01	0.004	0.11	0.75	0.05		
Chloroplast Type 5	Chl c_2	Alloxanthin	Crocoxanthin	βε-car				
Dinophysis acuminata VGO1063	0.07	1.62	0.08	0.20				
D. acuta VGO1065	0.09	1.35	0.07	0.18				
D. caudata VGO1064	0.07	1.38	0.05	0.19				
D. tripos VGO1062	0.09	1.32	0.05	0.15				
Chloroplast Type 6	Neo	Viola	Anth	Zea	Unk-car443-Lc	βε-car	ββ-car	Chl *b*
Lepidodinium chlorophorum Dino16EUH	0.09	0.08	0.01	0.01	0.17	0.04	0.04	0.73
L. chlorophorum RCC1488	0.14	0.16	0.01	0.05	0.16	0.03	0.05	0.56
L. chlorophorum RCC1489	0.14	0.12	0.02	0.06	0.14	0.03	0.05	0.57
L. chlorophorum BAH100ME	0.09	0.32	0.03	0.02	0.18	0.11	0.05	0.08

lates with other pigment composition have been reported (chl *b*, Watanabe et al. 1990; alloxanthin, Meyer-Harms & Pollehne 1998; acyloxyfucoxanthins, Bjørnland & Tangen 1979, Tengs et al. 2000).

We applied HPLC to review the pigment composition of dinoflagellates belonging to 6 orders of the division Dinophyta (Table S1). Members of the Gonyaulacales, Prorocentrales and Thoracosphaerales analysed contained exclusively the peridinin-containing chloroplast Type 1 (Table 6), which included around two-thirds of the studied species.

Recently, an exception to the common chloroplast Type 1 distribution in Gonyaulacales was observed in *Amylax buxus* and *A. triacantha*, where anucleated cryptophyte vestiges (probably *Teleaulax*-related) were detected (Koike & Takishita 2008). The order Dinophysiales comprises mostly heterotrophic dinoflagellates with only a reduced number of autotrophic species belonging to the genus *Dinophysis*. In

Table 6. Distribution (●) of pigment-based chloroplast types across Dinophyta taxa (class Dinophyceae)

Representative species	Chloroplast type 1	2	3	4	5	6
Dinophysiales						
Dinophysis acuminata	–	–	–	–	●	–
Gonyaulacales						
Alexandrium minutum	●	–	–	–	–	–
Gymnodiniales						
Gymnodinium catenatum	●	–	–	–	–	–
Karenia mikimotoi	–	●	–	–	–	–
Karlodinium veneficum	–	–	–	–	–	–
Takayama cf. *helix*	–	–	●	–	–	–
Lepidodinium chlorophorum	–	–	–	–	–	●
Peridiniales						
Heterocapsa sp.	●	–	–	–	–	–
Durinskia baltica	–	–	–	–	–	–
Galeidinium rugatum[a]	–	–	–	–	–	–
Kryptoperidinium foliaceum	–	–	–	●	–	–
Prorocentrales						
Prorocentrum lima	●	–	–	–	–	–
Thoracosphaerales						
Thoracosphaera heimii	●	–	–	–	–	–

[a]From Tamura et al. (2005)

this case, the cryptophyte chloroplast Type 5 was present, although the presence of a haptophyte-type plastid in *D. mitra* has been claimed (Koike et al. 2005). Such an important exception should be supported by evidence from HPLC pigment analysis, which has not been yet performed. Peridinin-containing species have never been reported in Dinophysiales. The order Peridiniales includes both peridinin-containing chloroplast Type 1 and the fucoxanthin-containing Type 4. The major pigment-based chloroplast diversity was observed in the Gymnodiniales, with 4 out of 6 chloroplast types defined here (Types 1, 2, 3 and 6). In addition, a few Gymnodiniales species have been reported to harbour endosymbiotic algae belonging to chloroplast Type 4 (*Gymnodinium quadrilobatum*, Horiguchi & Pienaar 1994) and Type 5 (*Amphidinium latum*, Horiguchi & Pienaar 1992) chloroplasts, but pigment analyses were not detailed in these studies. These findings support the description of Gymnodiniales as a heterogeneous order, as previously indicated by Saldarriaga et al. (2001)

Pigment-based chloroplast types and dinophyte phylogeny

Pigmentary groups in phytoplankton have been traditionally based on the occurrence of certain marker pigments (Jeffrey et al. 1999). However, in the present study we defined pigment-based chloroplast types to illustrate the fact that dinoflagellates include not only secondary but also tertiary plastids from different algal lineages. Dinoflagellates have acquired and lost their chloroplasts multiple times during their evolutionary history. The ability to acquire and maintain other eukaryotic plastids has led to the diversity of dinoflagellate plastids (Koike et al. 2005). Peridinin-pigmented dinoflagellates contain secondary plastids that appear to have undergone more plastid genome reduction than other eukaryotes. It is generally accepted that peridinin-containing dinoflagellate plastids are derived from red algae (Zhang et al. 1999), but whether they are secondary plastids equivalent to plastids of stramenopiles, haptophytes or cryptophytes, or they are tertiary plastids derived from one of the other secondary plastids, has not yet been completely resolved (Wang et al. 2008). The number of endosymbiotic events in dinoflagellates probably exceeds those in other known eukaryotes (Takishita et al. 2002).

Saldarriaga et al. (2004) proposed a scheme of the evolutionary history of dinoflagellates based on molecular trees of concatenated nuclear genes, morphological and palaeontological information. If we superimpose the pigment-based chloroplast types on that scheme (Fig. 3), it appears that most of the pigment diversity in dinoflagellates occurs in a certain group of dinoflagellate orders (Peridiniales, Dinophysiales, Gymnodiniales, Thoracosphaerales) sharing a common ancestor.

Peridiniales occupy a central position in the evolution of dinoflagellates and probably gave rise to other thecate taxa and also to Thoracosphaerales and Blastodiniales. Montresor et al. (2003) reported that Suessiales contain peridinin. *Karenia* and *Karlodinium* occupy an early divergent branch in many molecular studies and would also represent the first deviation of the chloroplast Type 1 (peridinin) among dinoflagellates.

Tertiary plastids in dinoflagellates

Karenia: chloroplast Type 2

Chloroplast Type 2 are considered tertiary plastids related to Type 7 haptophytes (Zapata et al. 2004): both contain chl c_2, c_3 and MGDG-chl c_2 [14:0/14:0] *Chrysochromulina*-type. However, the carotenoid composition of *Karenia* species detected in our study does not match any of the haptophyte pigment types

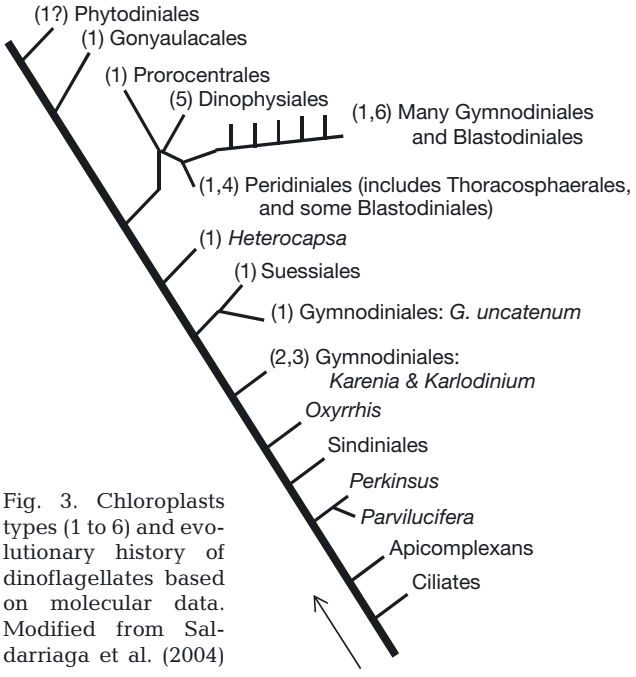

Fig. 3. Chloroplasts types (1 to 6) and evolutionary history of dinoflagellates based on molecular data. Modified from Saldarriaga et al. (2004)

described to date. In addition to Fuco, Hex-fuco and 4-keto-hex-fuco, 3 novel pigments were detected. One of these (peak 15) was tentatively identified as 4-keto-but-fuco; the other 2 pigments (peaks 13 and 22) shared both absorption spectra with the above mentioned 4-keto forms (Garrido et al. unpubl.). Pigment Type 2 embraces well-known HAB species belonging to the genera *Karenia* (i.e. *K. brevis*, *K. mikimotoi*, *K selliformis*). It is noteworthy that the particular fingerprint profile of *Karenia* species could be useful to detect their presence in monitoring programmes (Garrido et al. unpubl.).

Gyroxanthin diester was considered a marker pigment for *Karlodinium veneficum* (= *Gymnodinium galatheanum*) (see Bjørnland et al. 2000) and also for *Karenia brevis* (Bjørnland et al. 2003). However, a pigment with similar chromatographic properties has also been detected in the pelagophyceans *Pelagomonas calceolata* (Bjørnland et al. 2003), and tentatively *in Aureococcus anophageferens*, *Pelagococcus subviridis* (Zapata 2005) and several haptophytes (Zapata 2005). The occurrence of gyroxanthin in *Karlodinium* and *Karenia* is noticeable for its quantitative relevance, but it is not taxon specific (Garcés et al. 2006). The genus *Karenia* currently embraces 13 species (Guiry & Guiry 2010). Thus, the homogeneity in the pigment composition observed in the 4 species analysed here should be confirmed by the analysis of 9 additional species whose original or further description (see Table S5 in the supplement) did not include pigment data.

Karlodinium and *Takayama*: chloroplast Type 3

Chloroplast Type 3 lacks keto forms of acyloxy-fucoxanthin-derivatives, and the occurrence of both MGDG-chl c_2 and gyroxanthin diester is not a general trait. Pigment Type 3 groups the well-known HAB species *Karlodinium veneficum* (= *K. micrum*, *Gymnodinium galatheanum*). The genus *Karlodinium* currently includes 10 species (de Salas et al. 2008); however, the pigment composition of 5 of them has not yet been analysed (see Table S5). HPLC pigment analysis of *K. australe* (de Salas et al. 2005) indicates that this species contains Fuco and Hex-fuco as the main carotenoid pigments, with But-fuco present in trace amounts. *K. australe* did not produce gyroxanthin-diester, a pigment typical of *K. veneficum* (*K. micrum*, Bjørnland et al. 2000) and *Karenia* species (de Salas et al. 2004).

In a recent study, Bachvaroff et al. (2009) analysed pigment variability between *Karlodinium veneficum* strains and detected 2 GyrE compounds sharing its absorption spectra and molecular weight. They were noted as GyrE and *cis* GyrE isomers, not as esters with different fatty acid residues.

The marine dinoflagellate genus *Takayama* currently comprises 6 species (Table S5). *Takayama* cf. *helix* (VGO-341) contains an MGDG-chl c_2 pigment previously detected in *Emiliania huxleyi* (peak 53) and a second one also detected in *Prymnesium faveolatum* (peak 60). A different pigment profile was observed in *T. tasmanica*, which contains a GyrE-like pigment more polar than that detected in *Karenia umbella* (de Salas et al. 2004). In addition, *T. tasmanica* and *T.* cf. *helix* contain an MGDG-chl c_2, closely eluting to zeaxanthin, also detected in *Haptolina* (= C*hrysochromulina*) *hirta* and other *Chrysochromulina* species (Zapata et al. 2001, Seoane et al. 2009) recently reasigned to the new genus *Haptolina* (Edvardsen et al. 2011). However, the 2 *Takayama* species differ in that the occurrence of GyrE is restricted to *T. tasmanica*, and Hex-fuco is only detected in *T.* cf. *helix* (de Salas et al. 2003). The other 4 *Takayama* species have not yet been analysed for pigment composition: *T. acrotrocha, T. cladochroma*, *T. pulchella* (de Salas et al. 2003) and *T. tuberculata* (de Salas et al. 2008).

Durinskia and *Kryptoperidinium*: chloroplast Type 4

The dinoflagellate species included in this chloroplast type correspond with those described as bearing diatom endosymbionts: *Durinskia baltica* (= *Peri-*

dinium balticum) (Withers et al. 1977, Carty & Cox 1986) and *Kryptoperidinium foliaceum* (Jeffrey & Vesk 1976, Kite et al. 1988). Chl c_1 is the dominant chl *c* pigment in *K. foliaceum* and *D. baltica*; such a feature is also characteristic of *Pavlova* spp. (Van Lenning et al. 2003, Zapata et al. 2004), but it is unusual in diatoms (Stauber & Jeffrey 1988).

In addition to fucoxanthin and chl c_1 and c_2, Type 4 dinoflagellates contain minor chl *c*-like pigments first detected, respectively, in the pavlovophyceans *Pavlova gyrans* (Fawley 1989) and *Exanthemachrysis gayraliae* (Van Lenning et al. 2003). These pigments also occur in several chrysophytes (e.g. synurophyceans and chrysophyceans; Zapata 2005, Jeffrey & Wright 2006). Previous HPLC analysis of *Kryptoperidinium foliaceum* (Kempton et al. 2002, McEwan & Keeling 2004) did not detect these pigments. The presence of β,ψ-carotene in *K. foliaceum* and *Durinskia baltica* is a singular feature with no correspondence in Bacillariophyceae and pavlovophyceans (haptophyte pigment Type 2). However, βψ-car is a light-sensitive pigment, so its potential role as marker pigment is restricted to high light environments. Molecular analyses have traced the chloroplast origin of *Peridinium balticum* (= *D. baltica*) and *K. foliaceum* to a pennate diatom (Chesnick et al. 1997).

According to Imanian et al. (2010), the endosymbiont in these dinoflagellate species would be closely related to the pennate diatom genus *Nitzschia*. However, *Kryptoperidinium foliaceum* seems to have incorporated 2 exogenous plasmids. These authors proposed the term 'dinotom' to refer to the complex cell derived from this tertiary endosymbiosis. The nature of the endosymbiont is similar in *Durinskia cappensis* (Pienaar et al. 2007). In the dinoflagellates *Galeidinium rugatum* (Tamura et al. 2005) and *Peridinium quinquecorne* (Horiguchi & Takano 2006), the diatom plastid seems to be from a centric diatom (Takano et al. 2008) obtained by serial replacement of diatom endosymbionts. Whether this different chloroplast (centric versus pennate diatom) source is reflected in the pigment composition of *G. rugatum* and *P. quinquecorne* is still under debate.

Dinophysis: chloroplast Type 5

The genus *Dinophysis* includes both photosynthetic and non-photosynthetic (heterotrophic) species, and the former are known to possess cryptophyte-type plastids that contain the phycobilin pigment phycoerythrin (Vesk et al. 1996, Hewes et al. 1998, Hackett et al. 2003).

The origin of the *Dinophysis* spp. chloroplast was traced to *Teleaulax amphioxeia* (Janson 2004) and closely related to *Geminiphera cryophyla* in *Dinophysis* spp. from the Greenland Sea (Janson 2004, Minnhagen & Janson 2006). Most species belonging to the genus *Dinophysis* harbour chloroplasts of cryptophyte origin. Whether these chloroplasts are temporarily sequestered from the prey (kleptoplastids) or permanent is controversial. Considering both molecular and ultrastructural evidence, Garcia-Cuetos et al. (2010) indicated that the plastids in *D. acuminata* are permanent and originate from *Teleaulax* or another closely related cryptophyte genus. Koike et al. (2005) suggested the presence of a haptophyte-type plastid in *D. mitra*, although HPLC pigment data were not reported**.** If this is confirmed, it would be the first case of an armoured dinoflagellate containing a haptophyte-type chloroplast. The difficulty in culturing *Dinophysis* was a bottleneck for many decades to advance in basic studies of its biology (Park et al. 2006). At present, distinct species of *Dinophysis* have been cultured by different laboratories (*D. acuminata*: Park et al. 2006; *D. fortii*: Nagai et al. 2008; *D. caudata*: Nishitani et al. 2008a; *D. infundibulus*: Nishitani et al. 2008b; *D. tripos*: Rodríguez et al. 2012). However, our study represents the first HPLC pigment data from cultured *Dinophysis* species.

Lepidodinium chlorophorum: chloroplast Type 6

The genus *Lepidodinium* was recently revised (Hansen et al. 2007), and the previously named *Gymnodinium chlorophorum* was renamed as *L. chlorophorum*. The extant plastid in the dinoflagellate *L. viride* is most probably acquired by plastid replacement via tertiary endosymbiosis (reviewed by Delwiche 2007). This dinoflagellate possesses a green-pigmented plastid surrounded by 4 membranes. First evidence of pigment composition (W. W. C Gieskes pers. comm. to Elbrächter & Schnepf 1996) pointed out the presence of prasinoxanthin; as a consequence, the prasinophyte-origin of the chloroplast was assumed. Recent results indicate that the green plastids in *Lepidodinium* are derived from an alga belonging to core chlorophytes (Minge et al. 2010, Matsumoto et al. 2011), distinct from the ancient prasinophyceans. Our results show clearly that *L. chlorophorum* lacks prasinoxanthin. Moreover, the results obtained (see Table 2) also differ from other pigment patterns so far observed within the prasinophycean algae (Egeland et al. 1995, Latasa et al. 2004, Yoshii 2006, Garrido et al. 2009). The absence

(or traces) of lutein is noticeable, as is the occurrence of an unknown carotenoid (peak 42) with similar retention time as lutein in the HPLC system employed. Both differences in the UV-vis spectrum (Table 1) and chromatographic retention using a different HPLC method (Garrido et al. 2009) allowed distinction of the unknown pigment from lutein and other major pigments detected in Chlorophyta. The structural elucidation of this carotenoid (peak 42) is currently under investigation (Zapata et al. unpubl.).

Implications of dinoflagellate chloroplast types in biological oceanography and chemotaxonomy

The use of HPLC pigment analysis for inferring phytoplankton assemblages through marker pigments increased the interest for phytoplankton pigments in oceanography (revised by Jeffrey et al. 1997, 1999, Jeffrey & Wright 2006). The use of peridinin to map the contribution of dinoflagellates to total chl *a* is generally accepted due to the prevalence of peridinin-containing chloroplasts in photoautotrophic dinoflagellates. In fact, our study seems to validate this general approach, as peridinin was the characteristic marker pigment in two-thirds of the analysed species. However, this approach is prone to error if a single pigment algorithm is employed. For example, the contribution of dinoflagellates to total chl *a* has usually been obtained by using a fixed equation: [chl *a*] DINO = 1.5 [Peri] obtained from a single *Amphidinium* sp. isolate (see Letelier et al. 1993), which corresponds to Peri:chl *a* ratios of 0.67 (mass ratio) or 0.65 (molar ratio, where molar ratio is MW chl *a*:MW Peri = 0.97 × mass ratio). The contribution of dinoflagellates to total chl *a* assuming such a fixed factor will produce either an overestimation or subestimation, assuming the Peri:chl *a* range obtained in the present paper (0.54–2.06 molar ratio, Table 3). This range for Peri:chl *a* ratios agrees well with that obtained in field studies using the chemical taxonomy programme CHEMTAX (from 0.52 to 1.51, Table S6). CHEMTAX (see Mackey et al. 1996) has demonstrated its capacity for reconstructing phytoplankton assemblages from HPLC pigment data (Wright et al. 1996, Mackey et al. 1998, Higgins & Mackey 2000, Wright & van den Enden 2000, Rodríguez et al. 2003). This program relies upon (1) general information of major algal groups present in the study area and (2) an initial pigment to chl *a* ratio matrix basically obtained from the literature for relevant phytoplankton groups ('algal classes'), or even user-defined 'algal pigment classes' (Rodríguez et al. 2003). In this sense, the values shown in Tables 3 to 5 represent improved pigment ratios and define new 'chemotaxonomic categories' (i.e. '*Dinos* Types 1 to 6') which may be incorporated into CHEMTAX analysis.

The use of peridinin as a single marker pigment for dinoflagellates ignores the potential contribution of species from chloroplast Types 2 to 6, whose contribution to chl *a* would be assigned to other algal groups with similar pigment composition (Table S7). For example, the contribution of Hex-fuco-containing dinoflagellates to total chl *a* might be underestimated due to the presence of haptophytes sharing similar pigment composition. Some bloom-forming dinoflagellates (*Karenia brevis, K. mikimotoi, Karlodinium veneficum*) share several pigment markers with bloom-forming haptophytes (*Chrysochromulina* spp., *Emiliania huxleyi*, *Phaeocystis* spp.), but the specificity of chloroplast Type 2 allows us to discriminate *Karenia* species from Hex-fuco-containing haptophytes. Comparing molar pigment ratios of haptophytes from pigmentary groups 6, 7 and 8 (sensu Zapata et al. 2004) with Hex-fuco-containing dinoflagellates, the values chl c_3:chl *a*, chl c_2:chl *a*, chl c_3:chl c_2 and total chl *c*:total fuco are consistently higher in haptophytes relative to dinoflagellates of Types 2 and 3 (see Tables 3 & 5 in Zapata et al. 2004). The index MGDG-chl c_2:chl *a* in Type 2 and 3 dinoflagellates is an order of magnitude lower than the values obtained within Types 6, 7 and 8 haptophytes (see Zapata et al. 2001, 2004, Seoane et al. 2009).

Type 4 dinoflagellates show a pigment profile closely related to some diatoms (Zapata 2005) and pavlovophyceans Type 2 (e.g. *Pavlova gyrans*: chl c_2-like *P. gyrans* type). In addition, *Durinskia baltica* and *Kryptoperidinium foliaceum* contain βψ-car, which was claimed as the differential pigment in *K. foliaceum* (Kempton et al. 2002). However, βψ-car contents have been shown to be dependent on light intensity in *Tetraselmis suecica* (Garrido et al. 2009), so the marker quality of this pigment in other species needs confirmation under different light regimes. Type 5 dinoflagellates share the pigment profile with autotrophic cryptophytes. In consequence, it is not possible to discriminate both algal groups in natural samples. The occurrence of green dinoflagellates is easily detected by pigment analysis, as both the presence of chl *b* and the singular carotenoid profile are very characteristic. No other chl *b*-containing organism with similar pigment signature has been reported to date.

The importance of dinoflagellates in the picophytoplankton fraction, which have not been cultured yet, has been shown. For example, Latasa & Bidigare

(1998) found that often more than 50% (and up to 75%) of peridinin appeared in the <2 µm fraction, whereas on the basis of peridinin concentration, Wright et al. (2009) estimated that dinoflagellates accounted for 0 to 11% of picoplanktonic chl *a* in Antarctic waters. In addition, molecular sequences from many unknown dinoflagellates have been retrieved in open ocean samples (Moon-van der Staay et al. 2001, Lin et al. 2006). All of these facts underscore the necessity to isolate and cultivate new species of small dinoflagellates so that their pigment composition can be characterized, to advance our knowledge about the diversity of photosynthetic dinoflagellates in natural samples.

Acknowledgements. This work was supported by funds from the Xunta de Galicia, Spain (PGIDIT-06PXIB6021 43PR), from the Spanish Ministry of Science and Innovation (CSIC (200930I009) and from Instituto Español de Oceanografía (project CCIEO). This manuscript is a contribution from the Unidad Asociada IEO/CSIC 'Fitoplancton Tóxico'. We thank E. Campaña and N. Sanz for HPLC pigment analyses and I. Ramilo and A. Fernández-Villamarín for technical assistance with algal cultures.

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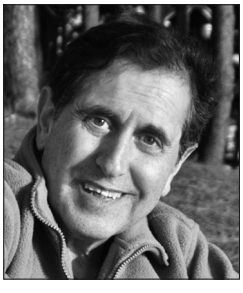
Editorial responsibility: Katherine Richardson, Copenhagen, Denmark

Submitted: October 25, 2011; Accepted: June 18, 2012
Proofs received from author(s): September 14, 2012

In Memoriam. While this paper was being reviewed, our colleague and friend Manuel Zapata passed away. He contributed greatly to the present state of knowledge of algal pigments (on their structures, methods of analysis, taxonomical distribution and use as signatures for characterizing natural phytoplankton communities). His HPLC method for algal pigments (Zapata et al. 2000; see Literature Cited) has become a standard procedure in marine laboratories around the world. 'Zapa', as his family and friends called him affectionately, left us an important body of knowledge and a special way to observe nature. We will always remember him.



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